SKIPANON FEDERAL CHANNEL
BIOASSAY
SEDIMENT QUALITY EVALUATION
REPORT

March 2004

Prepared by:
Tim Sherman

Reviewed by:
Mark Siipola

Portland District
Corps of Engineers
CENWP-EC-HR

ACRONYMS

Ag	Silver
As	Arsenic
Cd	Cadmium
CoC	Contaminate of concern
Cr	Chromium
CRM	Columbia River Mile
Cu	Copper
DMEF	Dredge Material Evaluation Framework
DQO	Data Quality Objectives
EPA	Environmental Protection Agency
GC	Gravity Core
Hg	Mercury
MDL	Method Detection Limit
MLLW	Mean Lower Low Water
MRL	Method Reporting Limit
Eohaustorius estuarius	Amphipod (crustacean) used in 10-day acute analyses
Neanthes arenaaceodentata	Marine polychaete worm used in 20-day growth analyses
Mytilus galloprovincialis	Mussel larva used in mortality and abnormality analyses
NES	Newly Exposed Surface
Ni	Nickel
NPL	National Priority List
PAH	Polynuclear Aromatic Hydrocarbon
Pb	Lead
PCB	Polychlorinated Biphenyl
PQL	Practical Quantitation Limit
QA/QC	Quality Assurance/Quality Control
RMT	Regional Management Team
Sb	Thallium
SL	Screening Limit
SRM	Skipanon River Mile
Superfund	An EPA-NPL contaminated site, scheduled for cleanup.
TEL	Threshold Effects Level
Tier II	Physical (a) & Chemical (b) analyses
Tier III	Bioassay & Bioaccumulation analyses
Tier IV	Special non-routine evaluation
TOC	Total Organic Carbon
TVS	Total Volatile Solids
USFWS	U. S. Fish & Wildlife Service
WDNR	Washington Department of Natural Resources
Zn	Zinc

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ABSTRACT

The Skipanon Channel empties into the west side of Young's Bay on the south side of the Columbia River at about river mile (CRM) 10.7 (Figure 1). The drainage basin for the Skipanon River is about 15 square miles. The river flows from Cullaby Lake, the source, to the Columbia River some 8-mile distance. Tidal effects extent upstream to Skipanon RM (SRM) 4.5

The Federal navigational project consists of a channel 30-feet deep and 200-feet wide, extending from the Columbia River navigational channel to SRM 2.0 (see Figure 1). At about SRM 1.5 the channel widens into a turning basin that extends to SRM 1.8, where the channel narrows again and proceeds to the bridge at SRM 2.0. Due to a lack of need for a deep channel, the channel is maintained to a depth of 16-feet, mean lower low water (MLLW), while the turning basin is maintained to 12-feet MLLW.

This evaluation was conducted following procedures set forth in the Inland Testing Manual, developed jointly by the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency to assess dredged material and the Dredge Material Evaluation Framework for the Lower Columbia River Management Area (DMEF). The DMEF is a regional manual developed jointly with regional EPA, Corps, Oregon Department of Environmental Quality and Washington Departments of Ecology and Natural Resources. This document is a guideline for implementing the Clean Water Act (40 CFR 230), Section 404 (b)(1). The screening levels used are those adopted for use in the DMEF, final November 1998. The DMEF tiered testing approach requires that material in excess of 20% fines and greater than 5% volatile solids, as well as any material with prior history or is suspected ("reason to believe") of being contaminated, be subjected to physical (Tier IIa) as well as chemical (Tier IIb) analyses. When the screening guidelines are exceeded, further characterization can be conducted following the DMEF Tier III protocol of bioassay analyses.

Due to DDT contamination detected in the 2001 sampling event, bioassay analyses were conducted on sediment collected during this sampling event.

A total of five (5) gravity-core sediment samples were collected along the Skipanon Federal channel and boat basin entrance channel June 24, 2003. All samples were submitted for physical analyses including total volatile solids and also were analyzed for metals (9 inorganic), total organic carbon, pesticides and polychlorinated biphenyls, phenols, phthalates, miscellaneous extractables and polynuclear aromatic hydrocarbon.

The physical analyses indicated mean values of 0.0% gravel, 23.9% sand (16.02%-31.80% range), and 76.1% silt/clay (68.20%-83.98% range), with 6.82% volatile solids (5.60%-7.75% range).

All chemical analyses results were below their respective DMEF, Tier IIb, screening level, except mercury (Hg) in sample SBIO-GC-04. Bioassay results did not indicate any problems with sample SBIO-GC-04, but did indicated a single-hit failure, according to the DMEF Tier III protocol, in sample SBIO-GC-03. The failure occurred in the *Mytilus* larval test on sediment represented by sample SBI0-GC-03 (see figure 2). This is the same area that exceeded DMEF, Tier IIb, screening levels for DDT in the 2001 sampling event, prompting this bioassay characterization.

With the exception of the sediment represented by sample SBI0-GC-03, the balance of the sediment represented by samples SBIO-GC-01, SBIO-GC-02 and SBIO-GC-04 is determined to be suitable for unconfined, in-water placement without further characterization. Sample SBIO-GC-03 sediment will need to be appropriately managed, if dredged. Further characterization under Tier IV of the DMEF could be considered, if the Regional Management Team (RMT) deems that the results of the Tier III were indeterminate. Factors to consider in this determination are:

- No DMEF chemical data screening levels (SL) were exceeded during this, 2003, sampling event.
- Was ammonia or other conditions a factor in failure?
- Similar unexplained *Mytilus* larval test failure (1998, Astoria East Boat Basin), in which no chemical exceedances were observed in simultaneous sediment analyses, bring into question the reliability of this test for this area.

A possible DMEF Tier IV test could include a sediment larval test using all appropriate Echinoderm and Bivalve larval in a simultaneous comparative test run, with follow-up interpretation.

INTRODUCTION

This report characterizes the sediment to be dredged at the Skipanon Federal channel and boat basin entrance channel for the purposes of dredging and disposal. The sampling and analysis objectives are stated in the Sampling and Analysis Plan (SAP June 2003), and are also listed below. This report will outline the procedures used to accomplish these objectives.

Sampling and Analysis Objectives

- To characterize sediments in accordance with the regional dredge material testing manual, the Dredge Material Evaluation Framework for the Lower Columbia River Management Area (DMEF).

- Collect, handle and analyze representative sediment, of the purposed dredging prism, in accordance with protocols and Quality Assurance/Quality Control (QA/QC) requirements.

- Characterize sediments to be dredged, for the evaluation of suitable disposal methods.

- Conduct Tier II a & b physical and chemical characterization, as well as, Tier III bioassay characterization for this sediment evaluation.

PREVIOUS STUDIES

Portland District routinely evaluates sediment from its projects on a 5-year rotational basis. Physical and chemical evaluation sampling was performed at the Skipanon Entrance Channel and Boat Basin starting in 1981, and continued in 1986 (as part of the proposed Coal Channel Project), 1991, 1996 & 2001. The results of these studies revealed the sediment, especially in Federal channel areas, to be predominately silt or sandy silt with volatile solids >5% (with some exceptions).

In the most recent (September 2001) sampling event, seven (7) samples were collected using a gravity core-sampling device. All samples were submitted for physical and chemical analyses, including TOC, 8 inorganic metals, PAHs, phthalates, phenols, misc. extractables, Pest/PCBs, pore-water TBT and one dioxin/furan. With the exception of sample SKIP-VC-04 (DDT at 7.1 ug/kg; S.L. = 6.9 ug/kg) none of the CoC exceeded the current DMEF screening levels. The material associated with sample SKIP-VC-04 could not be determined to be suitable for open water disposal, without further characterization at the DMEF Tier III level.

CURRENT SAMPLING EVENT/DISCUSSION

A total of five (5) samples were collected from the Skipanon Federal channel and boat basin entrance channel June 24, 2003 (see Figure 2 and Table 1).

Table 1. Sample Location Coordinates
(NAD 83, Oregon State Plane North)

SBIO-GC-01	46˚ 10 ’03.0”	SBIO-GC-02	46˚ 10’ 03.1”
	123˚ 55’ 14.4”		123˚ 55’ 11.0”
SBIO-GC-03	46˚ 10’ 3.9”	SBIO-GC-04	46˚ 10’ 11.9”
	123˚ 54’ 59.1”		123˚ 54’ 50.5”
SBIO-GC-R	46˚ 10’ 33.8”		
	123˚ 54’ 38.0”		

The samples were collected using a gravity-core sampling device (GC). All samples were submitted for physical analyses including total volatile solids (TVS) and were analyzed for metals (9 inorganic), total organic carbon (TOC), pesticides and polychlorinated biphenyls

(PCBs), phenols, phthalates, miscellaneous extractables, and polynuclear aromatic hydrocarbons (PAHs).

RESULTS

Physical and Volatile Solids (ASTM methods)

Four (4) samples and one (1) reference sample were submitted for physical and TVS analyses and data are presented in Table 2. Two (2) samples were classified as "sandy silt," two samples were classified as "silt with sand," and one sample was classified as "elastic silt with sand." Mean grain size for all the samples was 0.047 mm, with 0.0% gravel, 23.9% sand, and 76.1% fines. Volatile solids for all samples ranged from 5.60% to 7.75% with a mean value of 6.82%.

Metals (EPA method 6020/7471), Total Organic Carbon (EPA method 9060)

Four (4) samples and one (1) reference sample were submitted for testing and the data are presented in Table 3. The TOC ranged from 15,600 to 44,300 mg/kg (ppm) in the samples. Low levels of most metals were found, but most did not approach the DMEF screening level (SL), except for mercury in one sample (SBIO-GC-04), which exceeded the 0.41 mg/kg SL, with a reported level of 0.564 mg/kg.

Pesticides/PCBs (EPA method 8081A/8082), Phenols, Phthalates and Miscellaneous Extractables (EPA method 8270)

Four (4) samples and one (1) reference sample were tested for pesticides/PCBs and the data are presented in Table 4. No PCBs were found at the MDL in any of the samples. No pesticides (including DDT) were detected in any of the samples. Phthalate compounds were detected in all samples, with reported values well below the SL. Low-level contamination was present in the method blank for bis(2-Ethylhexyl)phthalate and in all samples. Phenols were detected in samples SBIO-GC-04 and SBIO-GC-R, but they were below their respective DMEF SLs (<8.5% and <7.8% respectively of the SL), as well.

Polynuclear Aromatic Hydrocarbons (EPA method 8270C)

Four (4) samples and one (1) reference sample were tested and the data are presented in Table 5. The "low molecular weight" PAHs were detected in all samples, with values below 2.2% of their respective DMEF SLs. "High molecular weight" PAHs were found in all samples, values ranged below 4.8% of their respective DMEF SLs.

Bioassay Analyses

Four (4) samples and one (1) reference sample were tested and the data are presented in Table 6. Biological testing is conducted to measure effects on organisms exposed to whole

sediment or overlying water column. Analyses were conducted using DMEF (1998) marine bioassay protocol.

1) Amphipod Test – This test involves exposing amphipods (species used *Eohaustorius estuarius*) to test sediment for ten (10) days and counting the surviving animals at the end of the exposure period. The amphipod test mortality was less than 30% over reference sediment, which is the acceptable level.

2) Neanthes Growth test – This test utilizes a polychaete (species used - *Neanthes arenaceodentata*), in a 20-day survival and growth test. The growth rate of organisms exposed to test sediments is compared to the average individual growth rate of organisms exposed to reference sediment. The reference test sediment did not meet performance criteria. In the absence of suitable reference sediment the test results were compared to the laboratory positive control sample, with positive performance.

3) Sediment Larval Test – This test monitors larval development of a suitable mollusk (species used – *Mytilus galloprovincialis*) or echinoderm species in the presence of test sediment. The test is run until the appropriate stage of development is achieved in a sacrificial seawater control. At the end of the test, larva from each test sediment exposure is examined to quantify abnormality and mortality. The seawater control has a performance standard of 30% combined mortality and abnormality. The reference sediment has a performance standard of 35% combined mortality and abnormality normalized to seawater control. The test sediment larval normalized combined mortality and abnormality exceeded 30% of the reference and showed statistical significant response relative to the reference sediment in sample SBIO-GC-03. This constitutes a "one-hit failure" (Sec 9.2.4 DMEF).

As a result of the "one hit-failure" in the larval test the material represented by this sample is considered unacceptable for in-water placement under Tier III testing protocol of the DMEF.

CONCLUSION

This evaluation was conducted following procedures set forth in the Inland Testing Manual, developed jointly by the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency to assess dredged material and the Dredge Material Evaluation Framework for the Lower Columbia River Management Area (DMEF). The DMEF is a regional manual developed jointly with regional EPA, Corps, Oregon Department of Environmental Quality and Washington Departments of Ecology and Natural Resources. This document is a guideline for implementing the Clean Water Act (40 CFR 230), Section 404 (b)(1). The screening levels used are those adopted for use in the DMEF, final November 1998. The DMEF tiered testing approach requires that material in excess of 20% fines and greater than 5% volatile solids, as well as any material with prior history or is suspected ("reason to believe") of being contaminated, be subjected to physical (Tier IIa) as well as chemical (Tier IIb) analyses. When the screening

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A possible DMEF Tier IV test could include a sediment larval test using all appropriate Echinoderm and Bivalve larval in a simultaneous comparative test run, with follow-up interpretation.

REFERENCES

1. U.S. Army Corps of Engineers, Portland District and Seattle District; U.S. Environmental Protection Agency, Region 10; Oregon Department of Environmental Quality; Washington State Department of Natural Resources and Department of Ecology. 1998 Final. Dredge Material Evaluation Framework for the Lower Columbia River Management Area.

2. U.S. Environmental Protection Agency and U.S. Army Corps of Engineers. February 1998. Evaluation of Dredged Material Proposed for Discharge in Inland and Near Coastal Waters - Testing Manual (referred to as the "Inland Testing Manual").

3. Clean Water Act, 40 CFR 230 (b)(1).

4. PSDDA. 1996. Puget Sound Dredged Disposal Analysis, Technical Information Memorandum, Testing, Reporting and Evaluation of Tributyltin Data in PSDDA and SMS Programs.

5. U.S. Army Corps of Engineers. September 2001. Sediment Sampling and Analysis Plan, Skipanon Channel and Boat Basin. Portland District.

6. U.S. Army Corps of Engineers. November 1996. Skipanon Channel Sediment Evaluation. Portland District.

7. U.S. Army Corps of Engineers. 1991. Skipanon River Sediment Evaluation. Portland District.

8. U.S. Army Corps of Engineers. November 1981. Skipanon Channel Sediment Evaluation. Portland District.

Table 2: Physical Analysis and Volatile Solids

Sampled June 24, 2003

Sample I.D.	Grain Size (mm)		Percent			
	Median	Mean	Gravel	Sand	Silt/Clay	Volatile Solids
SBIO-GC-01	0.021	0.049	0.00	21.07	78.93	6.66
SBIO-GC-02	0.035	0.050	0.00	30.65	69.35	7.36
SBIO-GC-03	0.013	0.042	0.00	16.02	83.98	5.60
SBIO-GC-04	0.017	0.049	0.00	31.80	68.20	7.75
*SBIO-GC-R	0.023	0.043	0.00	19.93	80.07	6.72
Mean	0.022	0.047	0.00	23.89	76.11	6.82
Minimum	0.013	0.042	0.00	16.02	68.20	5.60
Maximum	0.035	0.050	0.00	31.80	83.98	7.75

* SBIO-GC-R is sediment collected from a formerly tested, low-level contaminate area, used as a bioassay reference sediment.

Table 3: Inorganic Metals and TOC

Sample I.D.	As	Cd	Cr	Cu	Pb	Ni	Ag	Zn	Hg	TOC
	mg/kg (ppm)									
SBIO-GC-01	8.96	<0.71	22.8	52.3	20.3	18.2	<0.71	129	0.107	25500
SBIO-GC-02	7.51	<0.556	17.9	36.4	13.8	14	<0.556	90.1	0.102	44300
SBIO-GC-03	8.95	<0.63	25.2	56.9	22.5	19.9	<0.63	149	0.112	26400
SBIO-GC-04	9	0.852 J	32.3	46.2	22.5	20.3	<0.427	143	**0.564**	19700
*SBIO-GC-R	7.02	<0.418	18.7	32.2	13.7	15.4	<0.418	89.2	0.17	15600
Screening level (SL)	57	5.1	390	450		140	6.1	410	0.41	

J = Estimated value (reported values are above the MDL, but below the PQL).
Symbol (<) = Non-detect (ND) at the value listed (Method Detection Limit).
* SBIO-GC-R is sediment collected from a formerly tested, low-level contaminate area, used as a bioassay reference sediment.

Table 4: Pesticides, PCBs, Phenols, Phthalates & Misc. Extractables

	Pesticides				Phenol	Phthalates		
	µg/kg (ppb)							
Sample I.D.	4,4'-DDD	4,4'-DDE	4,4'-DDT	Total DDT	3&4 Methyl phenol	bis(2-Ethylhexyl) phthalate	Di-n-butyl phthalate	Diethyl phthalate
SBIO-GC-01	<2.8	<2.8	<2.8	ND	<56.7	62.2 B1	<28.3	<28.3
SBIO-GC-02	<2.4	<2.4	<2.4	ND	<44.8	35.2 J B1	<22.4	<22.4
SBIO-GC-03	<2.5	<2.5	<2.5	ND	<49.9	114 B1	<24.9	25.7 J
SBIO-GC-04	<1.92	<1.92	<1.92	ND	56.9 J	44 B1	<19.4	<19.4
SBIO-GC-R	<1.63	<1.63	<1.63	ND	52.3 J	22.5 J B1	21.1 J	<16.3
Screen level (SL)				6.9	670	8300	5100	1200

J = Estimated value (reported values are above the MDL, but below the PQL).
B1 = Low-level contamination was present in the method blank (reported level was < 10 times blank concentration).
Symbol (<) = Non-detect (ND) at the value listed (Method Detection Limit).
* SBIO-GC-R is sediment collected from a formerly tested, low-level contaminate area, used as a bioassay reference sediment.

Table 5: Polynuclear Aromatic Hydrocarbons (PAHs) Low Molecular Weight Analytes

Polynuclear Aromatic Hydrocarbons (PAHs) Low Molecular Weight Analytes µg/kg (ppb)								
Sample I.D.	**Acenaphthene**	**Acenaphthylene**	**Anthracene**	**Fluorene**	**2-Methyl naphthalene**	**Naphthalene**	**Phen-anthrene**	**Total Low PAHs**
SBIO-GC-01	5.41 J	3.36 J	8.54	<2.83	<7.09	4.77 J	14.7	36.78
SBIO-GC-02	<2.24	<2.24	4.42 J	<2.24	<5.6	3.21 J	7.02	14.65
SBIO-GC-03	5.84	<2.49	10.5	7.46	<6.23	5.63 J	25.5	54.93
SBIO-GC-04	4.44	9.87	11.2	9.52	<4.84	12	32.2	79.23
SBIO-GC-R	2.01 J	4.1	4.32	3.09 J	<4.07	6.7 J	12.8	33.02
Screen level (SL)	500	560	960	540	670	2100	1500	5200

Symbol (<) = Non-detect (ND) at the value listed (Method Detection Limit)
J = Estimated value (reported values are above the MDL, but below the PQL).
* SBIO-GC-R is sediment collected from a formerly tested, low-level contaminate area, used as a bioassay reference sediment.

Table 5 cont'd: Polynuclear Aromatic Hydrocarbons (PAHs) High Molecular Weight Analytes

Polynuclear Aromatic Hydrocarbons (PAHs) High Molecular Weight Analytes µg/kg (ppb)										
Sample I.D.	**Benzo(a)-anthracene**	**Benzo-fluro-anthenes**	**Benzo-(g,h,i)-perylene**	**Chrysene**	**Pyrene**	**Benzo(a)-pyrene**	**Indeno-(1,2,3-cd)-pyrene**	**Dibenz(a,h) anthracene**	**Fluor-anthene**	**Total High PAHs**
SBIO-GC-01	20.3	43.5	20.4	32.7	55.5	21.9	15.8	<3.72	47.1	260.92
SBIO-GC-02	8.94	<5.6	<2.24	13.6	16.1	<2.94	<2.94	<2.94	16	71.3
SBIO-GC-03	25.8	61.6	23.3	37.6	43.6	30.9	17.7	<3.27	38	281.77
SBIO-GC-04	29.7	65.6	31.8	34.1	47.7	43.4	28.3	<2.54	34.7	317.84
SBIO-GC-R	13.7	28.3	14.4	14.8	27.1	18.3	14.4	<2.14	22	155.14
Screen level (SL)	1300	3200	670	1400	2600	1600	600	230	1700	12000

J = Estimated value (reported values are above the MDL, but below the PQL).
Symbol (<) = Non-detect (ND) at the value listed (Method Detection Limit).
* SBIO-GC-R is sediment collected from a formerly tested, low-level contaminate area, used as a bioassay reference sediment.

Table 6: Summary of One- and Two-Hit Failures for the Indicated Tests

	Eohaustorius	***Neanthes ****	***Mytilus***	***Two-Hit Rule Failure***
SBIO-GC-01	No	No	No	No
SBIO-GC-02	No	No	No	No
SBIO-GC-03	No	No	Yes	No
SBIO-GC-04	No	No	No	No
* Reference sediment did not meet performance criteria				

See Appendix A (page 18) for additional information on Bioassay Analyses.

Figure 1: Skipanon Channel & Boat Basin Vicinity Map

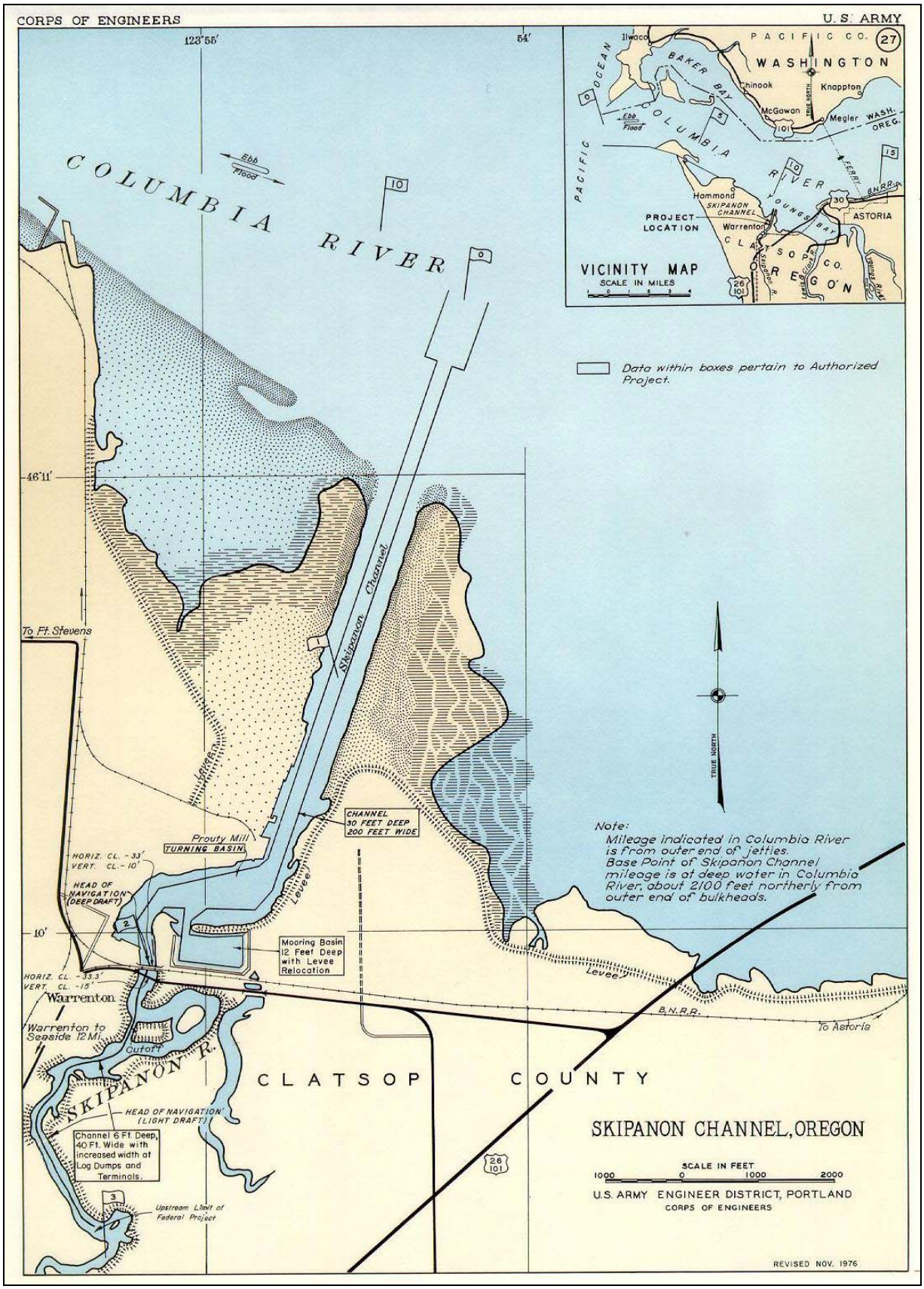
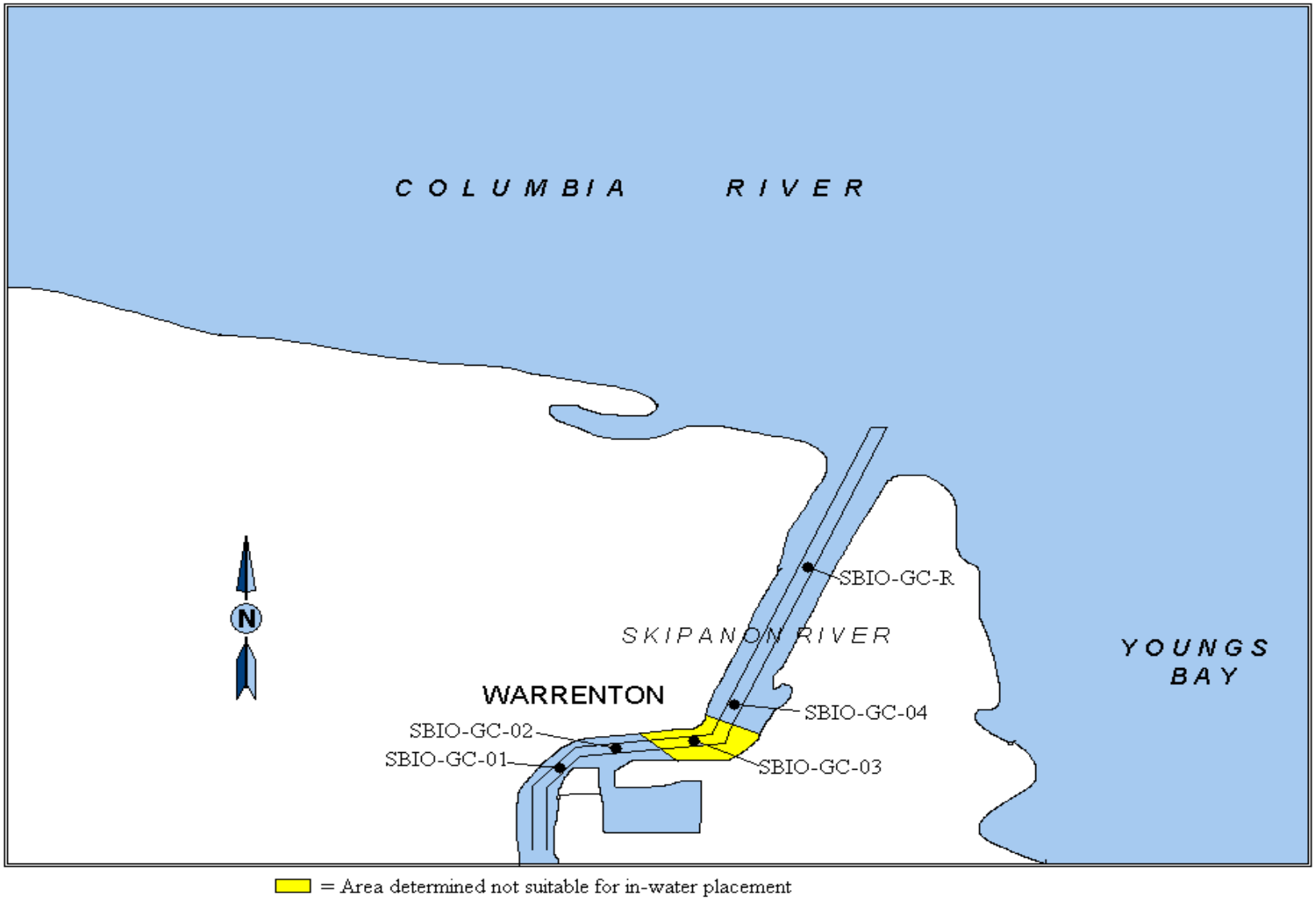


Figure 2: Skipanon Federal Entrance Channel, Sediment Sampling Station Locations



Skipanon Channel & Boat Basin Sampling Event Pictures

Appendix A

NORTHWESTERN AQUATIC SCIENCES
A Division of NAS Associates, Inc
P.O. Box 1437, Newport, Oregon 97365 • (541) 265-7225 Fax:(541) 265-2799 • contact@nwaquotic.com

August 29, 2003

Mr. Tim Sherman

U.S. Army Corps of Engineers
Portland District
333 SW 1st Portland, OR 97208

Dear Mr. Sherman:

Enclosed are two copies each of NAS test reports 694-1, -2, and -3, for 10-day *Eohaustorius,* 20-day *Neanthes* survival and growth, and *Mytilus* larval tests, respectively, that we recently completed for you.

Data interpretation was conducted as described in 1) "Dredged Material Evaluation and Disposal Procedures, A User's Manual for the Puget Sound Dredged Disposal Analysis (PSDDA) Program," February 2000 (U.S. Army Corps of Engineers, Seattle District; U.S. Environmental Protection Agency, Region 10, Washington Department of Natural Resources, Washington Department of Ecology), and 2) "Dredged Material Evaluation Framework, Lower Columbia River Management Area," November 1998 (U.S. Army Corps of Engineers, Northwestern Division; U.S. EPA, Region 10; Washington Department of Ecology, Oregon Department of Environmental Quality, and Washington Department of Natural Resources).

Analyses of the toxicity test data for Tests No. 694-1 *(Eohaustorius* test), 694-2 *(Neanthes* test), and 694-3 *(Mytilus* larval test) for dredged material disposal in Puget Sound according to the DMMP and DMEF "single-hit failure" rules are presented in the individual test reports. The table below summarizes the "single-hit failure" results as applied to nondispersive disposal (DMMP) and DMEF guidelines, and adds interpretation according to the "two-hit failure" guidelines. It should be noted that the results from the *Neanthes* test are subject to agency approval, since the reference sediment failed performance criteria in that test.

Summary of one- and two-hit rule failures for the indicated marine sediments.

	Eohaustorius	***Neanthes ****	***Mytilus***	***Two-Hit Rule Failure***
SBIO-GC-01	No	No	No	No
SBIO-GC-02	No	No	No	No
SBIO-GC-03	No	No	Yes	No
SBIO-GC-04	No	No	No	No
* Reference sediment did not meet performance criteria				

Only one sediment, SB 10-GC-03, showed a "one-hit" failure, in the *Mytilus* larval test. For both DMMP and DMEF guidelines, "two-hit failures" occur when any two tests do not show "single-hit failures," but show significantly different responses from the reference sediment (and less than 70% of the mean reference sediment growth rate for the *Neanthes* test). There were no such significant responses in the *Eohaustorius* or *Neanthes* tests, so there were no "two-hit failures."

Thank you for the opportunity to work with you on this project. If you have any questions, feel free to call Dick Caldwell or me at 541-265-7225, or mailto:mredmond@nwaquatic.com.

Sincerely,

Michele S. Redmond
Project Manager, Marine Sediments

Cc: Tom Boyden, STL

Report

of

Test No. 694-1

Eohaustorius estuarius 10-Day Sediment Toxicity Test of Marine Sediments

Submitted to

U.S. Army Corps of Engineers
Portland District
333 SW 1st
Portland, OR 97208

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

August 25, 2003

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 694-1
Title: *Eohaustorius estuarius* 10-day sediment toxicity test of marine sediments
Protocol: NAS-XXX-EE4, February 20, 1992. Rev.2 (Feb. 12, 2001). Based on: Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995), with modifications as specified by the Dredged Material Management Program (DMMP, formerly Puget Sound Dredged Disposal Analysis Program or PSDDA).

STUDY MANAGEMENT

Study Sponsor: Severn Trent Laboratories, Inc., STL Seattle, 5755 8th St. East, Tacoma, WA 98424, and U.S. Army Corps of Engineers, Portland District, 333 SW 1st, Portland, OR 97208
Sponsor's Study Monitor: Mr. Tom Boyden (STL) and Mr. Tim Sherman (COE).
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, Oregon 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: M.S. Redmond, M.S., Proj. Mngr./ Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R_S. Caldwell, Ph.D., Sr. Aq. Toxicol. G.J. Irissarri, B.S., Aq. Toxicol.; G.A. Buhler, B_S., Aq. Toxicol.; G.C. Hayes, B.S., Sr. Tech.; W.T. Montgomery, A.A., Tech.
Study Schedule:
Test Beginning: 7-11-03, 1155 hrs.
Test Ending: 7-21-03, 1100 hrs.
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3 814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Control Sediment: Control sediment (NAS Sample #8436F) was collected from the *Eohaustorius estuarius* amphipod collection site in lower Yaquina Bay, Oregon, on 7-7-03. Interstitial salinity was 31.5‰. The sediment was sieved through 0.5 mm stainless steel screen and stored at 4°C in the dark.
Test Sediments: Four unidentified marine test sediments and one reference sediment were tested. Details follow:

NAS Sample No.	8422F	8423F	8424F	8425F	8426F
Sample Description	SBIO-GC-01	SBIO-GC-02	SBIO-GC-03	SBIO-GC-04	SBIO-GC-R
Collection Date	6-24-03	6-24-03	6-24-03	6-24-03	6-24-03
Receipt Date	6-27-03	6-27-03	6-27-03	6-27-03	6-27-03
Interstitial Salinity (‰)	17.0	15.0	17.5	20.0	19.5

Storage: Samples were stored at 4°C in the dark.
Treatments: The samples were homogenized by mixing with stainless steel implements.

TEST WATER

Source: Yaquina Bay, Oregon
Date of Collection: 7-9-03
Water Quality:Salinity 28.0‰, pH 8.1
Pretreatment: Filtered to ≤ 0.40 um, salinity adjusted with MilliQ® deionized water, aerated.

TEST ORGANISMS

Species: *Eohaustorius estuarius,* amphipod
Age: adult
Source: Yaquina Bay, Oregon
Acclimation: Amphipods were collected on 7-7-03 at interstitial water quality conditions of 15°C and 19.0‰. They were acclimated to test salinity and held for four days before addition to the test. Average test conditions during this time were: temperature, 15.2 ± 0.2°C; dissolved oxygen 8.3 ± 0.2 mg/L; pH, 8.0 ± 0.1; and salinity, 27.2 ± 0.5‰. The photoperiod was constant light.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.
Test Chambers: 1 L covered borosilicate glass beakers
Test Volumes: 175 ml of test or control sediment; 950 ml total volume.
Replicates/Treatment: 5 (plus one water quality replicate, and one additional sacrificial replicate used to measure interstitial water ammonia-N and sulfide on day 0.)
Salinity adjustment: none
Organisms/Treatment: 100 (20/replicate); all beakers received test organisms except those sacrificed on day 0.
Water Volume Changes per 24 hr: None.
Aeration: Yes, at least 2 cm above the sediment surface.
Feeding: None.
Acceptance Criteria: Results are valid if mean control mortality does not exceed 10%, and does not exceed 20% in any one control replicate.
Performance Criteria: For DMMP testing, the reference sediments must have ≤20% mean mortality over the negative control sediment.
Effects Criteria: 1) survival after 10 days, 2) number of survivors reburied, and 3) daily emergence of amphipods from the test sediments. Death is defined as no visible appendage movement or response to tactile stimulation. Unrecovered animals at the end of the exposure period were considered dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, salinity, and pH, were measured in the water quality replicate test chamber daily. Total dissolved sulfide and total ammonia-N were measured in the overlying water of the water quality replicate test chamber on days 0 and 10. Interstitial ammonia-N, sulfide, pH, and salinity were measured in a sacrificial beaker on test day 0 and from the water quality beaker on test day 10. Interstitial water samples were obtained by centrifugation, or by settling in the case of very sandy sediments.Total soluble sulfide and total ammonia-N were measured using Hach test kits based on the methylene blue (EPA Method 3762) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. Unionized ammonia-N was computed using "Unionized Ammonia Calculator", v1.0 (Dr. Landon Ross, Florida Department of Environmental Protection). The photoperiod was constant light.

DATA ANALYSIS METHODS

The percent amphipod mortality, percent of surviving amphipods failing to rebury at the end of the test, and percent total effective mortality were determined from the final observations according to the formulas:

Percent Mortality = 100 x ([initial amphipods - surviving amphipods]/initial amphipods)

Percent Survivors not Reburied = 100 x ([surviving amphipods - number survivors reburied]/surviving amphipods)

Percent Total Effective Mortality = 100 x ([initial amphipods - surviving amphipods]+ [surviving amphipods - number survivors reburied])/initial amphipods

Another endpoint was the sum of observed daily sediment emergence events in a test beaker throughout the test. Control and treatment means and standard deviations for the biological endpoints described above and for

water quality data were computed using Microsoft EXCEL 2000. Percent mortality, in each test sediment, was compared against that in the control and in the reference sediment. An arcsine square root transformation was performed on percentage data before analysis. The software used for statistical comparisons was BioStat (Beta Y.4. l (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, Onesample T-test, Mann Whitney test, or Rankit Analysis was conducted, at the 0.05 level of significance.

PROTOCOL DEVIATIONS

1. Ammonia was not measured in bulk sediments as recommended by the DMMP program ("Ammonia and amphipod toxicity testing," DMMP clarification paper 4-29-02). However, day zero interstitial values (maximum 42 mg/L total ammonia-N, 0.144 mg/L un-ionized ammonia) were well below the values listed in the clarification paper where sample purging might be considered (60 mg/L total ammonia, 0.8 mg/L unionized ammonia). The maximum un-ionized ammonia value (0.144 mg/L) was also well below the concentration where an ammonia reference toxicant test would be needed (0.4 mg/L).
2. In two beakers on day 5, and in most beakers on day 10, aeration had ceased and needed to be reestablished, but dissolved oxygen measurements were not taken in all affected beakers. Those beakers for which oxygen readings were taken on day ten had acceptable oxygen concentrations (minimum 7.6 mg/L, or 92% saturation).
3. Sediments were not placed under a nitrogen atmosphere as required if there is head space in the sample containers and storage is >2 weeks before testing (in this case a maximum of 17 days). Because sediments arrived in thin flexible plastic bags ("trash bags"), it was considered impractical to purge with nitrogen. There was little air space in the bags.

REFERENCE TOXICANT TEST

A reference toxicant test with cadmium as $CdCl_2$ • 2½ H_20 was performed to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. Reference toxicant test raw data and the applicable control chart are included in Appendix II.

Test No.: 999-1640

Reference Toxicant and Source: $CdC1_2$ •2 ½H_20, Mallinckrodt Lot No. TNZ, 1.0 mg/ml stock prepared 4-2-03

Test Date: 7-11-03

Dilution Water Used: Yaquina Bay, Oregon, seawater, 12.0 ‰

Result: 96-hr LC50, 5.17 mg/L Cd. This result is within the laboratory's control chart warning limits (0.00 - 5.59 mg/L).

RESULTS AND DISCUSSION

Observations of overlying water quality parameters during the test are listed in Table I. The individual water quality measurements are located in the raw data (Appendix II).

All observations of standard water quality parameters were within ASTM specified ranges (Table 1). Dissolved sulfide was not detected in the bioassay water. Total ammonia-N concentrations ranged from <0.5 mg/L to 24 mg/L (<0.014 mg/L to 1.089 mg/L un-ionized ammonia).

Interstitial total ammonia-N concentrations ranged from <2.5 mg/L to 42 mg/L, with a maximum un-ionized ammonia concentration of 0.144 mg/L (Table 2).

Table 1. Summary of overlying water quality conditions during tests of the amphipod, *Eohaustorius estuarius*, exposed to marine sediments.

Parameter	Mean ±SD	Minimum	Maximum	N
Temperature (°C)	15.3 ± 0.3	14.8	16.0	66
Dissolved oxygen (mg/L)	7.9 ± 0.3	7.1	8.4	66
Salinity (‰)	27.8 ± 0.5	27.0	29.0	66
PH	8.1 ± 0.2	7.0	8.5	66
Dissolved sulfide (mg/L)	---	<0.02	<0.02	12
Total ammonia-N (mg/L)	---	<0.5	24	12
Unionized ammonia (mg/L)	---	<0.014	1.089	12

Table 2. Summary of interstitial water quality conditions during tests of the amphipod, Eohaustorius estuarius, exposed to marine sediments.

Parameter	Mean SD	Minimum	Maximum	N
Salinity (‰)	27.2 ± 0.9	25.0	29.0	12
PH	6.8 ± 0.4	6.2	7.4	12
Dissolved Sulfide (mg/L)	---	<0.5	<0.5	12
Total Ammonia-N (mg/L)	---	<2.5	42	12
Un-ionized ammonia (mg/L)	---	---	0.144	12

Table 3 shows the effects of test sediment exposures on mortality and emergence. Reburial data was not tabulated, as all surviving amphipods reburied.

Table 3. Means and standard deviations (n=5) of sediment emergence and 10-day percent mortality of *Eohaustorius estuarius* exposed to sediments.

Sample description	Emergence (no./replicate)[1]	Percent mortality
Control (NAS #8436F)	0.0 ± 0.0	1.0 ± 2.2
SB 10-GC-R (NAS #8426F)	0.2 ± 0.4	19.0 ± 11.9*
SB 10-GC-01 (NAS #8422F)	3.4 ± 2.6	15.0 ± 7.1*
SB10-GC-02 (NAS #8423F)	3.4 ± 5.1	12.0 ± 4.5*
SB 10-GC-03 (NAS #8424F)	1.0 ± 1.2	14.0 ± 11.4*
SB 10-GC-04 (NAS #8425F)	0.4 ± 0.9	24.0 ± 9.6*

' Daily emergence counts include all amphipods observed on or above the sediment surface whether living or dead.
*Mortality was significantly greater than that in the control sediment ($p<0.05$).
‡Mortality was significantly greater than that in the reference sediment ($p<0.05$).

The test met the acceptability criterion (≤90%) for mean control survival; mean survival in the control was 99.0% (Table 3). In addition, replicate control survival was 100%, 100%, 100%, 100%, and 95%; therefore, all replicates met the replicate acceptability criterion (>80% in any one replicate). The reference toxicant test result (5.17 mg/L) was within the laboratory's control chart warning limits (0.00 to 5.59 mg/L).The reference sediment met the DMMP performance 'criterion of <20% mean mortality over the negative control sediment (SBIO-GC-R mortality = 19.0%, negative control mortality = 1.0%).

The test control sediment acceptance criterion and reference sediment performance standard for survival were met. Positive control performance was within the laboratory's acceptance limits. It is concluded, therefore, that the test has developed fully acceptable data for use in making management decisions.

Data analysis and interpretation (Table 4) were first conducted based on guidelines from the "Dredged Material Evaluation and Disposal Procedures, A User's Manual for the Puget Sound Dredged Disposal Analysis (PSDDA) Program," February 2000 (U.S. Army Corps of Engineers, Seattle District; U.S. Environmental Protection Agency, Region 10, Washington Department of Natural Resources, and Washington Department of Ecology). For a test sediment to fail under these guidelines, under the single hit rule, the mean test mortality must be >20% absolute over the mean negative control response, > 10% (dispersive) or >30% (nondispersive) absolute over the mean reference sediment response, and statistically different ($\alpha = 0.05$) from the reference sediment.

Data interpretation is similar under the guidelines in "Dredged Material Evaluation Framework, Lower Columbia River Management Area," November 1998 (U.S. Army Corps of Engineers, Northwestern Division; U.S. EPA, Region 10; Washington Department of Ecology, Oregon Department of Environmental Quality, and Washington Department of Natural Resources). For a test sediment to fail under these guidelines, under the single hit rule, the mean test mortality must be >20% absolute over the mean negative control response, >30% absolute over the mean reference sediment response, and statistically different ($\alpha = 0.05$) from the reference sediment. This is identical to the "nondispersive" one-hit rule under the DMMP guidelines.

Table 4.Single-hit criteria interpretation of *Eohaustorius* test data for dredged material disposal in Puget Sound, based on DMMP/PSDDA (USACE 2000) guidelines. The "nondispersive rule" criteria are comparable to the DMEF (USACE 1998) guidelines.

Sample description	Percent Mortality	Significantly different from Ref. Sed. At α = 0.05?	Percent difference over negative control	Percent difference over reference sediment	Failure under 1-hit dispersive rule?	Failure under 1-hit nondispersive rule?
Control (NAS #8436F)	1.0 ± 2.2	---	---	---	---	---
SB 10-GC-R (NAS 48426F)	19.0 ±11.9	---	18.0	---	---	---
SB 10-GC-01 (NAS #8422F)	15.0 ± 7.1	NO	14.0	-4.0	NO	NO
SB I O-GC-02 (NAS #8423F)	12.0 ± 4.5	NO	11.0	-7.0	NO	NO
SB 10-GC-03 (NAS #8424F)	14.0 ±11.4	NO	13.0	-5.0	NO	NO
SB 10-GC-04 (NAS #8425F)	24.0 ± 9.6	NO	23.0	5.0	NO	NO

Percent mortality in all test sediments and in the reference sediment was significantly higher than that in the control. Percent mortality in one test sediment (SB 10-GC-04) was more than 20% greater than that in the control. However, in no test sediment was percent mortality significantly higher than that in the reference sediment, and therefore none failed under the "single-hit" rule as defined by the DMMP or DMEF guidelines.

Report

of

Test No. 694-2

Juvenile *Neanthes* 20-Day Sediment Toxicity Test of Marine Sediments

Submitted to

U.S. Army Corps of Engineers
Portland District
333 SW 1st
Portland, OR 97208

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

August 25, 2003

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 694-2
Title: Juvenile *Neanthes* 20-day sediment toxicity test of marine sediments
Protocol: NAS-XXX-NA4, June 20, 1990. Rev.3 (10-31-97). Based on: Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995), with modifications as specified by the Dredged Material Management Program (DMMP, formerly Puget Sound Dredged Disposal Analysis Program or PSDDA).

STUDY MANAGEMENT

Study Sponsor: Severn Trent Laboratories, Inc., STL Seattle, 5755 8th St. East, Tacoma, WA 98424, and U.S. Army Corps of Engineers, Portland District, 333 SW 1st, Portland, OR 97208
Sponsor's Study Monitor: Mr. Tom Boyden (STL) and Mr. Tim Sherman (COE).
Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, Oregon 97365.
Test Location: Newport Laboratory.
Laboratory's Study Personnel: M.S. Redmond, M.S., Proj. Mngr./ Study Dir.; L.K. Nemeth, M.B.A., QA Officer; R_S. Caldwell, Ph.D., Sr. Aq. Toxicol; G.J. Irissarri, B.S., Aq. Toxicol. G.A. Buhler, B_S., Aq. Toxicol.; G.C. Hayes, B.S., Sr. Tech.; W.T. Montgomery, A.A., Tech.
Study Schedule:
Test Beginning: 7-11-03, 1100 hrs.
Test Ending: 7-31-03
Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3 814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).
Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Control Sediment: Control sediment (NAS Sample #8437F) was collected from lower Yaquina Bay, Oregon, on 7-7-03. The sediment was sieved through 0.5 mm stainless steel screen and stored at 4°C in the dark.
Interstitial salinity was 31.5‰.
Test Sediments: Four unidentified marine test sediments and one reference sediment were tested. Details follow:

NAS Sample No.	8422F	8423F	8424F	8425F	8426F
Sample Description	SBIO-GC-01	SBIO-GC-02	SBIO-GC-03	SBIO-GC-04	SBIO-GC-R
Collection Date	6-24-03	6-24-03	6-24-03	6-24-03	6-24-03
Receipt Date	6-27-03	6-27-03	6-27-03	6-27-03	6-27-03
Interstitial Salinity (‰)	17.0	15.0	17.5	20.0	19.5

Storage: Samples were stored at 4°C in the dark.
Treatments: The samples were homogenized by mixing with stainless steel implements.

TEST WATER

Source: Yaquina Bay, Oregon
Date of Collection: 7-9-03
Water Quality: Salinity 28.0‰, pH 8.1
Pretreatment: Filtered to ≤ 0.40 um, salinity adjusted with MilliQ® deionized water, aerated.

TEST ORGANISMS

Species: *Neanthes arenaaceodentata,* marine polychaete worm
Age: 2-3 week post-emergence juveniles
Initial wt.: 0.84 mg
Source: Laboratory cultures at the Department of Biology, California State University, Long Beach, California. Worms were received on 7-8-03.
Acclimation: Conditions during acclimation were: temperature, 19.7 ± 1.1°C; salinity, 32.0 ± 1.6‰; dissolved oxygen, 6.7 ± 0.6 mg/L; pH 7.7 ± 0.5. Photoperiod was constant light.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 1 L covered borosilicate glass beakers
Test Volumes: 175 ml of test or control sediment; 950 ml total volume.
Replicates/Treatment: 5 (plus one water quality replicate)
Salinity adjustment: None
Organisms/Treatment: 25 (5/replicate)
Water Volume Changes: One third of the seawater in each beaker was replaced every third day.
Aeration: Provided using a 1-ml glass pipette with the tip 3-4 cm below the water surface. Air was bubbled at a low rate (150-300 ml/min) so as not to disturb the sediment surface.
Feeding: Animals were fed 40 mg TetraMarine® per beaker every other day.
Acceptance Criteria: Results are valid if mean control survival is at least 80% (NAS Protocol No. NAS-XXX-NA4). DMMP requires control sediment mortality of ≤10% and a growth rate of >038 mg/individual/day.
Performance Criteria: For DMMP testing, the reference sediment mortality should be ≤20% with a growth rate of ≥80% that of the negative control sediment.
Effects Criteria: 1) survival after 20 days, 2) average individual biomass, and 3) average individual growth rate. Death is defined as no visible appendage movement or response to tactile stimulation. Missing worms are considered dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, salinity, and pH, were measured in the water quality replicate test chamber daily. Total dissolved sulfide and total ammonia-N were measured in the overlying water of the water quality replicate test chamber on days 0 and 10. Interstitial ammonia-N, sulfide, pH, and salinity were measured in a sacrificial beaker on test day 0 and from the water quality beaker on test day 10. Interstitial water samples were obtained by centrifugation, or by settling in the case of very sandy sediments. Total soluble sulfide and total ammonia-N were measured using Hach test kits based on the methylene blue (EPA Method 3762) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. Unionized ammonia-N was computed using "Unionized Ammonia Calculator", v1.0 (Dr. Landon Ross, Florida Department of Environmental Protection). The photoperiod was constant light.

DATA ANALYSIS METHODS

The percent amphipod mortality, percent of surviving amphipods failing to rebury at the end of the test, and percent total effective mortality were determined from the final observations according to the formulas:

Percent survival = 100 x (no. of surviving worms/initial number of worms)
Individual biomass = total dry wt. of worms/number of surviving worms weighed
Individual growth rate = (individual biomass - the initial dry wt.)/the number of test days

The means and standard deviations were then calculated for each treatment level. The statistical software employed for these calculations was Microsoft Excel 2000. Individual growth rate in each test sediment was compared against that in the control sediment and against that in the reference sediment. The software used for statistical comparisons was BioStat (Beta v.4.1 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. Following determination of normality and homogeneity of variances, a one-tailed

Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.05 level of significance.

PROTOCOL DEVIATIONS

1.Four test sediments had an interstitial salinity <20.0‰and were not adjusted on day -1 as described in the PSEP (1995) procedures. At test termination, all interstitial salinities were 29.0 - 30.0 ‰.

2. The protocol-specified temperature of 20.0 f 1.0 °C was exceeded on two instances on day 9 (maximum 21.3°C).
3. Sediments were not placed under a nitrogen atmosphere as required if there is head space in the sample containers and storage is >2 weeks before testing (in this case a maximum of 17 days). Because sediments arrived in thin flexible plastic bags ("trash bags"), it was considered impractical to purge with nitrogen. There was little air space in the bags.

REFERENCE TOXICANT TEST

The reference toxicant test is a standard multi-concentration toxicity test using cadmium as $CdCl_2$•2½H_20, to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix II.

Test No.: 999-1642
Reference Toxicant and Source: $CdC1_2$•2½H_20, Mallinckrodt, Lot No. TNZ, 1.0 mg/ml stock prepared 4-2-03
Test Date: 7-11-03
Dilution Water Used: Yaquina Bay, Oregon, seawater; 28.0 ‰
Result: The 96-hr LC50 was 7.25 mg Cd/L. This result is within the laboratory's control chart warning limits (4.20 - 11.4 mg Cd/L).

RESULTS AND DISCUSSION

Observations of overlying water quality parameters during the test are shown in Table 1. The individual water quality measurements are located in the raw data (Appendix II).

Except as noted above, all the measurements of standard water quality parameters were within protocol-specified ranges (Table 1). Sulfides were not detected in the overlying bioassay water. Total ammonia-N concentrations ranged from <0.5 mg/L to 15 mg/L (<0.020 mg/L to 0.850 mg/L un-ionized ammonia).

Table 1. Summary of overlying water quality conditions during tests of the amphipod, *Eohaustorius estuarius*, exposed to marine sediments.

Parameter	Mean ±SD	Minimum	Maximum	N
Temperature (°C)	20.3 ±0.3	19.7	21.3	48
Dissolved oxygen (mg/L)	6.6 ± 0.6	5.7	8.2	48
Salinity (‰)	27.8 ± 0.7	26.0	29.5	48
PH	7.9± 0.1	7.7	8.2	48
Dissolved sulfide (mg/L)	---	<0.02	<0.02	12
Total ammonia-N (mg/L)	---	<0.5	15	12
Unionized ammonia (mg/L)	---	<0.020	0.850	12

Table 2 shows the effects of test sediment exposures on survival and growth of *Neanthes*. The test met the acceptability criterion (>90%) for control survival; mean survival in the control was 92.0%. The individual growth rate in the controls averaged 1.31 mg/day/worm. This meets DMMP recommendation for4 a minimum growth rate of 0.72 mg/day for *Neanthes*. The average initial weight of worms was 0.84mg, within

the recommended range of 0.5 - 1.0 mg. The reference sediment included in the study did not meet the performance standard requirement that mortality in the reference sediment should be ≤20% (mortality in reference sediment SB 10-GC-R was 28.0%), or the growth rate criterion. According to DMMP criteria, the mean individual growth rate in the reference sediment should be ≥80% of the mean individual growth rate in the control sediment. Control growth rate was 1.31 mg/day/worm, and that in the reference sediment was 0.54 mg/day/worm, 41.2% of the control growth rate.

Table 2. Means and standard deviations (n=5) of percent survival, individual dry weight, and individual growth rate of *Neanthes* exposed for 20 days to marine sediments, and final interstitial salinity values.

Sample description	Percent Survival (20-days)	Individual dry wt. (mg)	Individual growth rate (mg/day/worm)	Final interstitial salinity(ppt)
Control (NAS #8437F)	92.0 ± 11.0	27.0 ± 2.6	1.31 ± 0.13	29.0
SBIO-GC-R (NAS #8426F)	72.0 ± 17.9	11.7 ± 5.0	0.54 ± 0.25 *	29.5
SBIO-GC-01 (NAS #8422F)	76.0 ± 8.9	18.9 ± 6.3	0.90 ± 0.32 *	29.5
SBIO-GC-02 (NAS #8423F)	80.0 ± 20.0	21.8 ± 2.5	1.05 ± 0.13 *	29.5
SBIO-GC-03 (NAS #8424F)	84.0 ± 21.9	20.3 ± 4.7	0.97 ± 0.23 *	29.0
SBIO-GC-04 (NAS #8425F)	80.0 ± 20.0	12.2 ± 4.9	0.57 ± 0.25 *	30.0

* Individual growth rate was significantly less than that in the control sediment ($p<0.05$).
‡Individual growth rate was significantly less than that in the reference sediment ($p<0.05$).
Reference sediment did not meet performance standards.

Survival and growth in the control were acceptable, and the reference toxicant (positive control) result was within the control chart limits for this species. However, the reference sediment failed to meet performance criteria, so the value of the data analysis and interpretation, which follows is up to the agencies concerned. Statistical comparison of growth rate in the test sediments with that in the control has been provided in the event that concerned agencies permit the substitution of the control sediment for the reference sediment. Data interpretation was conducted on the basis of the reference sediment comparison, as directed in the guidance documents cited below.

Data analysis and interpretation were first conducted based on guidelines from the "Dredged Material Evaluation and Disposal Procedures, A User's Manual for the Puget Sound Dredged Disposal Analysis (PSDDA) Program," February 2000 (U.S. Army Corps of Engineers, Seattle District; U.S. Environmental Protection Agency, Region 10, Washington Department of Natural Resources, Washington Department of Ecology). For a test sediment to fail under these guidelines, under the single hit rule, the mean individual growth rate must be <80% of the mean negative control growth rate, and <70% (dispersive) or <50% (nondispersive) of the mean reference sediment growth rate, and statistically different ($\alpha = 0.05$) from the reference sediment.

Data interpretation is similar under the guidelines in "Dredged Material Evaluation Framework, Lower Columbia River Management Area," November 1998 (U.S. Army Corps of Engineers, Northwestern Division; U.S. EPA, Region 10; Washington Department of Ecology, Oregon Department of Environmental Quality, and Washington Department of Natural Resources). For a test sediment to fail under these guidelines, under the single hit rule, the mean individual growth rate must be <80% of the mean negative control growth rate, <50% of the mean reference sediment growth rate, and statistically different ($\alpha = 0.05$) from the reference sediment. This is identical to the "nondispersive" one-hit rule under the DMMP guidelines.

All test sediments and the reference sediment showed significantly lower individual growth rates when compared to the control. Mean individual growth rates in all test and reference sediments were <80% of the mean negative

control individual growth rate, except in SB 10-GC-02 (80.2%). However, individual growth rates in the test sediments were in no case significantly lower when compared to the reference sediment, and in fact, individual growth rates in all test sediments were higher than that in the reference sediment. Therefore, the test sediments did not fail under the single-hit rule as defined by the DMMP or DMEF guidelines.

Table 3. Single-hit criteria interpretation of *Neanthes* juvenile infaunal growth test data for dredged material disposal in Puget Sound, based on DMMP/PSDDA (USACE 2000) guidelines. The "nondispersive rule" criteria are comparable to the DMEF (USACE 1998) guidelines.

Sample description	Individual Growth rate (mg/day)	Significantly different from Ref. Sed. at $\alpha = 0.05$?*	Percent of negative control	Percent of reference sediment value	Failure under 1-hit dispersive rule?	Failure under 1-hit nondispersive rule?
Control (NAS #8436F)	1.31 ± 0.13	---	---	---	---	---
SB 10-GC-R (NAS 48426F)	0.54 ± 0.25	---	41.2	---	---	---
SB 10-GC-01 (NAS #8422F)	0.90 ± 0.32	NO	68.7	167	NO	NO
SB I O-GC-02 (NAS #8423F)	1.05 ± 0.13	NO	80.2	194	NO	NO
SB 10-GC-03 (NAS #8424F)	0.97 ± 0.23	NO	74.0	180	NO	NO
SB 10-GC-04 (NAS #8425F)	0.57 ± 0.25	NO	43.5	106	NO	NO

* Reference sediment did not meet performance standards.

Report

of

Test No. 694-3

Larval Sediment Toxicity Test with
Mytilus galloprovincialis

Submitted to

U.S. Army Corps of Engineers
Portland District
333 SW 1st
Portland, OR 97208

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

August 29, 2003

SCIENCES TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 694-3
Title: *Mytilus galloprovincialis* larval sediment toxicity test of marine sediments.
Protocol: NAS-XXX-CG4/MG4, June 20, 1990. Rev. 2, Feb. 10, 1997. Based on: Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments (PSEP 1995), with modifications as specified by the Dredged Material Management Program (DMMP, formerly Puget Sound Dredged Disposal Analysis Program or PSDDA).

STUDY MANAGEMENT

Study Sponsor: Severn Trent Laboratories, Inc., STL Seattle, 5755 8th St. East, Tacoma, WA 98424, and U.S. Army Corps of Engineers, Portland District, 333 SW 1st, Portland, OR 97208
Sponsor's Study Monitor: Mr. Tom Boyden (STL) and Mr. Tim Sherman (COE).
Testinp, Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, Oregon 97365. Test Location: Newport Laboratory.
Laboratory's Study Personnel: M.S. Redmond, M.S., Proj. Mngr.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, MBA, QA Officer; R.S. Caldwell, Ph.D., Sr. .Aq. Toxicol.; G.C. Hayes, B.S, Sr. Tech.; W.T. Montgomery, A.A., Tech.
Study Schedule:

Test Beginning: 7-16-03, 1545 hrs.

Test Ending: 7-18-03, 1605 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.
Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792). Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Four unidentified marine test sediments and one reference sediment were tested. Details follow:

NAS Sample No.	8422F	8423F	8424F	8425F	8426F
Sample Description	SBIO-GC-01	SBIO -GC-02	SBIO -GC-03	SBIO -GC-04	SBIO -GC-R
Collection Date	6-24-03	6-24-03	6-24-03	6-24-03	6-24-03
Receipt Date	6-27-03	6-27-03	6-27-03	6-27-03	6-27-03
Interstitial Salinity (‰)	17.0	15.0	17.5	20.0	19.5

Storage: Samples were stored at 4°C in the dark.
Treatments: The samples were homogenized by mixing with stainless steel implements.

TEST WATER

Source: Yaquina Bay, Oregon
Date of Collection: 7-16-03
Water Quality: Salinity 27.0 ‰, pH 7.7
Pretreatment: Filtered to ≤0.40 pin, salinity-adjusted with MilliQ® deionized water, aerated.

TEST ORGANISMS

Species: *Mytilus galloprovincialis*
Age: 1.9 hrs post-fertilization
Source: Mussels were purchased from Carlsbad Aquafarm, Carlsbad, CA, and received on 7-11-03.

Acclimation: Adult animals were held under ambient outdoor conditions in Yaquina Bay, Oregon seawater. Average water quality conditions in the five days prior to testing were: temperature, 19.0°C; dissolved oxygen, 8.4 mg/L; pH, 8.0; and salinity, 32.0 ‰.
Source of Gametes: 2 females, 1 male.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix 1) for a more detailed description of the test procedures used in this study.

Test Chambers: 1 L covered borosilicate glass beakers
Test Volumes: 18 g of test sediment with 900 ml of test water added. Sediment was allowed to settle for the normal period of 4 hours as specified in the protocol.
Replicates/Treatment: 5 (plus a 6th water quality replicate).
Sediment Salinity Adjustment: None required.
Initial Concentration of Test Organisms: 24.4/ml
Water volume changes per 24 hours: None
Volume of Subsamples Taken for Counting: 10 ml
Aeration: Yes
Feeding: None
Acceptance Criteria: The percent normal larvae in the seawater control must be 2:70% at the end of the test..
Performance Criteria: For DMMP projects, the combined mortality and abnormality in the reference sediments must be ≤35% of the seawater control value.
Effects Criteria: The effects criteria used were: 1) mortality; 2) abnormal development to the fully-shelled stage; and 3) the combined mortality/abnormality endpoint. Normal development is defined as transformation to the fully shelled, straight-hinged, D-shaped prodissoconch I stage. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed, and 3) the number of normal larvae observed. The results were expressed as: 1) percent abnormality; 2) percent mortality; 3) combined percent mortality and abnormality; and 4) normalized (to the seawater control) a) percent mortality and b) combined percent mortality and abnormality.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, salinity, and pH, were measured in the water quality replicate test chamber daily. Total dissolved sulfide and total ammonia-N were measured in the overlying water of the water quality replicate test chamber on days 0 and 2. Total soluble sulfide and total ammonia-N were measured using I-lach test kits based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis. The photoperiod was 14:10, L: D.

DATA ANALYSIS METHODS

All three standard endpoints, percent abnormal, percent combined mortality/abnormality, and percent mortality have occasionally been computed both with, and without, normalization for the seawater control. Endpoints in this report have been computed according to the following formulas:

PABN (Percent Abnormality) = 100*(A/T)
PABND (Combined Percent Mortality/Abnormality) = 100*((I-N)/I)
PMORT (Percent Mortality) = 100*((I-T)/I)
NPM (Normalized Percent Mortality) = 100*(1-(T/TS))
NCMA (Normalized Combined Percent Mortality/Abnormality) = 100*(1-(N/NS))

where the following are counts per 10 ml subsample:

N = normal larvae counted
A = abnormal larvae counted
T = N+A (total larvae counted)
I = number of inoculated embryos (from average of zero time counts)
TS = average of total larvae counted in seawater controls
NS = average of normal larvae counted in seawater controls

The means and standard deviations were then calculated for each treatment level. The statistical software employed for these calculations was Microsoft Excel 2000. The number normal in each test sediment was compared against that in the control and in the reference sediment. NCMA was reported, but analyses were conducted on the number normal due to the statistical software's inability to analyze negative numbers in the NCMA endpoint. The software used for statistical comparisons was BioStat (Beta v.4.1 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Approximate T-test, One-sample T-test, Mann Whitney test, or Rankit Analysis was conducted at the 0.10 level of significance.

PROTOCOL DEVIATIONS

Sediments were not placed under a nitrogen atmosphere as required if there is head space in the sample containers and storage is >2 weeks before testing (in this case a maximum of 22 days). Because sediments arrived in thin flexible plastic bags ("trash bags"), it was considered impractical to purge with nitrogen. There was little air space in the bags.

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper as $CuS0_4$-$5H_2O$ to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix II. The reference toxicant test is conducted following EPA/600/R-95/136 (Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, August 1995).
Test No.: 999-1641
Reference Toxicant and Source: Copper as $CUS0_4$-$5H_20$, Argent Lot #0195, 1.0 mg/ml stock prepared 10-2-02.
Test Date: 7-16-03
Dilution Water Used: Yaquina Bay, Oregon, seawater
Result: 48-hr EC50, 10.5 pg/L Cu. This result is within the laboratory's control chart warning limits (9.09 to 12.4 pg/L Cu).

RESULTS AND DISCUSSION

Observations of water quality parameters during the test are listed in Table 1. Individual water quality measurements are located in the raw data (Appendix II).

The measurements of standard water quality parameters were all within protocol specified ranges (Table 1). Sulfides were not detected in the overlying bioassay water (detection limit 0.02 mg/L). Total ammonia-N ranged from <0.5 mg/L to 2.5 mg/L.

Table 1. Summary of water quality conditions during the tests of mussel, *Mytilus galloprovincialis*, larvae exposed to marine sediments.

Parameter	Mean ± SD	Minimum	Maximum	N
Temperature (°C)	15.7 ± 0.3	15.2	16.1	18
Dissolved Oxygen (mg/L)	7.7 ± 0.3	7.4	8.2	18
Salinity (ppt)	27.4 ± 0.4	27.0	28.0	18
pH	7.8± 0.2	7.5	8.2	18
Dissolved Sulfide (mg/L)	---	<0.02	<0.02	12
Total Ammonia-N (mg/L)	---	<0.5	2.5	12

Means and standard deviations of the normalized combined percent mortality and abnormality (NCMA) endpoint for sediments are summarized in Table 2. Detailed data organized by sample and replicate, including the larval counts, for all calculated endpoints are given in Appendix 11. A total of six test replicate subsamples were recounted (QC counts) as a check on the acceptability of the initial counts (Appendix 11). In all instances the QC counts were close (coefficients of variation from 2 to 10 for counts of normal larvae) to the initial counts and were considered acceptable.

Table 2. Means and standard deviations (n=5) of responses of mussel, *Mytilus galloprovincialis*, larvae exposed to marine sediments.

Sample Description	Normalized combined percent mortality & abnormality (NCMA)
Seawater control	0.0 ± 7.4
SBIO-GC-R (NAS #8426F)	30.0 ± 9.2*
SBIO-GC-O1 (NAS #8422F)	40.7 ± 5.3 *‡
SBIO-GC-02 (NAS #8423F)	29.3 ± 6.6*
SBIO-GC-03 (NAS #8424F)	67.1 ± 22.0*‡
SBIO-GC-04 (NAS #8425F)	40.6 ± 4.2 *‡

* Number normal was significantly less than that in the seawater control ($p<0.10$).
‡ Number normal significantly less than that in the reference sediment ($p<0.10$).

The test met the control acceptance criterion of ≥70% normal in the seawater control; the control percent normality was 79.2%. The reference sediment met the performance criterion of ≤35% combined mortality and abnormality when normalized to the seawater control (NCMA for SB 10-GC-R = 30.0%; see Table 2). Since control acceptance criteria, reference sediment performance criteria, and positive control acceptance criteria were all met, it is concluded that the test has developed acceptable data for use in making management decisions.

Data analysis and interpretation (Table 3) were first conducted based on guidelines from the "Dredged Material Evaluation and Disposal Procedures, A User's Manual for the Puget Sound Dredged Disposal Analysis (PSDDA) Program," February 2000 (U.S. Army Corps of Engineers, Seattle District; U.S. Environmental Protection Agency, Region 10, Washington Department of Natural Resources, Washington Department of Ecology). For a test sediment to fail under these guidelines, under the single hit rule, the mean NCMA for a test sediment must be >20%, 15% (dispersive) or 30% (nondispersive) absolute over the mean reference sediment NCMA, and statistically different ($\alpha = 0.10$) from the reference sediment NCMA.

Data interpretation is similar under the guidelines in "Dredged Material Evaluation Framework, Lower Columbia River Management Area," November 1998 (U.S. Army Corps of Engineers, Northwestern Division; U.S. EPA, Region 10; Washington Department of Ecology, Oregon Department of Environmental Quality, and Washington Department of Natural Resources). For a test sediment to fail under these guidelines, under the single hit rule, the mean NCMA for a test sediment must be >20%, 30% absolute over the mean reference sediment NCMA, and statistically different ($\alpha = 0.10$) from the reference sediment NCMA. This is identical to the "nondispersive" one hit rule under the DMMP guidelines.

Table 3. Single-hit criteria interpretation of *Mytilus* larval sediment bioassay data for dredged material disposal in Puget Sound, based on DMMP/PSDDA (USACE 2000) guidelines. The "nondispersive rule" criteria are comparable to the DMEF (USACE 1998) guidelines.

Sample description	Normalized combined mortality & abnormality (%)	Significantly different from reference sediment at $\alpha = 0.10$?	Percent difference (greater) from reference sediment value	Failure under 1-hit dispersive rule?	Failure under 1-hit nondispersive rule?
Seawater control	0.0 ± 7.4	---	---	---	---
SBIO-GC-R (NAS #8426F)	30.0 ± 9.2	---	---	---	---
SBIO-GC-0I (NAS #8422F)	40.7 ± 5.3	YES	10.7	NO	NO
SBIO-GC-02 (NAS #8423F)	29.3 ± 6.6	NO	-0.7	NO	NO
SBIO-GC-03 (NAS #8424F)	67.1 ± 22.0	YES	37.1	YES	YES
SBIO-GC-04 (NAS #8425F)	40.6 ± 4.2	YES	10.6	NO	NO

The number normal in all test sediments and in the reference sediment was significantly lower than that in the control. The number normal in all test sediments except SBIO-GC-02 was significantly lower than that in the reference sediment. All test sediments had NCMA values of >20%. However, only in test sediment SB 10-GC-03 was the NCMA (67.1%) >15% and >30% above the reference sediment NCMA (30.0%). SB10-GC-03 therefore failed under the "single-hit" rule as defined by the DMMP or DMEF guidelines.